

SUPPLEMENTARY INFO

An Evaluation of the Efficacy and Safety of TAMIXAM®, Based on Hyaluronic Acid and Tamarind Seed Extract, for the Esoph-ageal Mucosal Protection from Acid Insult

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Table S1. Results of the Macroscopical Evaluation (0=absence of erythema, 1=slight erythema, 2= well-defined erythema, 3= moderate erythema, 4= severe erythema or eschar formation)

Day	Hamster treated	Before Treatment	After treatment	Mean	Hamster control	Before Treatment	After treatment	Mean
1	7	0	0	0	1	0	0	0
1	8	0	0	0	2	0	0	0
1	9	0	0	0	3	0	0	0
2	7	0	0	0	1	0	0	0
2	8	0	0	0	2	0	0	0
2	9	0	0	0	3	0	0	0
3	7	0	0	0	1	0	0	0
3	8	0	0	0	2	0	0	0
3	9	0	0	0	3	0	0	0
4	7	0	0	0	1	0	0	0
4	8	0	0	0	2	0	0	0
4	9	0	0	0	3	0	0	0
5	7	0	0	0	1	0	0	0
5	8	0	0	0	2	0	0	0
5	9	0	0	0	3	0	0	0
6	7	0	0	0	1	0	0	0
6	8	0	0	0	2	0	0	0
6	9	0	0	0	3	0	0	0
7	7	0	0	0	1	0	0	0
7	8	0	0	0	2	0	0	0
7	9	0	0	0	3	0	0	0
8	7	0	0	0	1	0	0	0
8	8	0	0	0	2	0	0	0
8	9	0	0	0	3	0	0	0
9	7	0	0	0	1	0	0	0
9	8	0	0	0	2	0	0	0
9	9	0	0	0	3	0	0	0
10	7	0	0	0	1	0	0	0
10	8	0	0	0	2	0	0	0
10	9	0	0	0	3	0	0	0

Table S2 – Housing conditions for animals *Oral mucosa irritation test*

Animals (breed)	Syrian Hamsters
Identification	Animals will be individually identified with a progressive number starting from 1.
Weight	> 150 g
Enrollment and acclimatization	On the enrollment day, animals will be individually weighed, identified and the veterinarian or qualified personnel will perform a clinical visit to verify the health status of animals. The enrolled animals will be acclimatized for at least 5 days. The actual acclimatization period will be reported in the Final Report.
Food	A commercially available, GLP compliant, rodents' diet will be provided <i>ad libitum</i> . Feed label will be reported in the Final Report.
Water	Drinking water will be provided <i>ad libitum</i> , delivered through an automatic watering system and/or drinking bottles.
Housing	Animals will be individually housed in polycarbonate cages with wire covers, identified by indicating the study code, the group code and animal number.
Environmental	<ul style="list-style-type: none"> • The recommended temperature range for rodents is 20-24°C ± 1.5°C. The room temperature will be monitored by a data logger. • The relative humidity will not be lower than 45 ± 5% or higher than 65 ± 5%. The room relative humidity will be monitored by a data logger. • The light cycle will be controlled using an automatic timer (12 hours light, 12 hours dark).
Animals health status	Animals health status will be checked daily and the clinical observations will be reported on the experimental register.
Veterinary Care	In the unlikely event that an animal should become injured, ill, or moribund, care will be conducted in accordance with current veterinary medical practice. If warranted for humane reasons, euthanasia will be conducted in accordance with the UE Recommendation 2007/526/CE, the ISO 10993-2, UE Directive 2010/63, enforced by Italian Legislative Decree Number 26 of March 4 th , 2014.

Table S3 – Housing conditions for animals *Skin sensitization tests – guinea pig maximization tests (GPMT)*

Hartley guinea pigs	
Acclimatization period	Before the start of veterinary experimental procedures, animals should be acclimatized at least 5 days.
Food	<p>A commercially available, GLP compliant, rodents' diet will be provided <i>ad libitum</i>. Feed label will be reported in the Final Report.</p> <p>A daily check of the food presence will be done and reported on the experimental register.</p>

Water	Drinking water will be provided <i>ad libitum</i> , delivered through drinking bottles. A daily check of the water presence will be done and reported on the experimental register.
Housing	Animals will be group housed in Noryl cages, labelled with the study code, experimental group and the animal identification number.
Environmental	<ul style="list-style-type: none"> • The recommended temperature range for guinea pigs is 18-24°C. The room temperature will be monitored by a data logger. • The relative humidity will not be lower than 30% or higher than 70%. The room relative humidity will be monitored by a data logger. • The light cycle will be controlled using an automatic timer (12 hours light, 12 hours dark).
Veterinary Care	In the unlikely event that an animal should become injured, ill, or moribund, care will be conducted in accordance with current veterinary medical practice. If warranted for humane reasons, euthanasia will be conducted in accordance with the UE Recommendation 2007/526/CE, the ISO 10993-2, UE Directive 2010/63, enforced by Italian Legislative Decree Number 26 of March 4 th , 2014.

Table S4 Cytotoxicity test

Grade	Reactivity	Reactivity description
0	none	Discrete amount of intracellular granulations; no cell lysis
1	light	not more than 20% of the cells are round, approximately adhesions and without intracellular granulations; occasional presence of lysis cells
2	slight	no more than 50% of cells are round and without granulation intracellular; not extensive presence of cell lysis and empty areas between cells.
3	moderate	no more than 70% of the cell layer contains round or lised cells.
4	severe	Almost complete destruction of the cell layer

Table S5 Oral mucosa irritation test

The reactions that appeared in the treated area were assessed macroscopically on the basis of the following rating scale

Mean score	Description of response
0	None
1 to 4	Minimal
5 to 8	Slight
9 to 10	Moderate

Table S6 - Histological preparations were evaluated using the following rating scale:

Epithelium		Leukocyte infiltration		Vascular congestion		Edema	
Normal	0	Absent	0	Absent	0	Absent	0
Degeneration or flattening cells	1	Minimal – less than 25	1	Minimal	1	Minimal	1
Metaplasia	2	Slight – 26-50	2	Slight	2	Slight	2
Focal erosion	3	Moderate 51-100	3	Moderate	3	Moderate	3
Generalised erosion	4	Marked – greater than 100	4	Marked with vessl rupture	4	Marked	4

Table S7 – Irritation Index

Mean score	Description of response
0	None
1-4	Minimal
5-8	Slight
9-11	Moderate
12-16	Severe

Table S8 – Scoring System Skin sensitization tests - guinea pig maximization test (GPMT)

Erythema: erythema and excoriations	Description of response
Absence of erythema 0	0
Discrete or irregular erythema 1	1
Moderate and confluent erythema 2	2
Intense erythema and/or swelling 3	3